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ARTICLE

Pollen morphology of the genus *Impatiens* L. (Balsaminaceae) and its systematic implications

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ABSTRACT Pollen morphology of 18 species from the genus *Impatiens* collected from different localities in Nilgiris, Tamil Nadu was evaluated using scanning electron microscopy (SEM) during the period of February 2017 to November 2019. From the observation of pollen it was found to be structurally monad; prolate, sub-prolate and prolate-spheroidal in equatorial view; and circular, rectangular, triangular, quinquangular, elliptic and quadrangular in polar view. The apertures varied from dicolpate, tricolpate to tetracolpate. The main ornamentation type was reticulate in most of the species except *Impatiens fruticosa* which showed echinate ornamentation. The variations in pollen structure within the species were useful for the identification and classification of the genus *Impatiens*. The high structural diversity renders important taxonomic value for species differentiation.

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Introduction

The genus *Impatiens* L. is a highly diverse genus of the family Balsaminaceae representing with over 1000 species distributed in tropical and subtropical regions (Janssens et al. 2018). In India, there are more than 210 species within Himalaya and the Western Ghats as the two major diversity centres (Shajitha et al. 2016). The Western Ghats is one of the richest areas in the world considering the distribution of species of *Impatiens* where about 103 species of *Impatiens* are endemic (Bhaskar 2012). They usually occur in wet and moisture conditions from sea level to 4000 m altitude, valleys, along streams, while some species tolerate drier habitats (Yu et al. 2015).

The vegetative morphology of *Impatiens* is conserved, always having a glandular-toothed leaf, fleshy semi-succulent stem and reflected in the hypervariable floral morphology in which the spurred sepal and the lateral petals show extreme variability (Fig. 1-2). High levels of convergent evolution on flower morphology are probably the main reason why it has been so difficult in the past to divide the genus into natural groups based on macro-morphological data only (Janssens et al. 2012).

Representatives of the genus are known for their convergent phenotypic adaptation often making it extremely difficult to divide the genus into natural groupings using morphological data (Janssens et al. 2018). Grey-Wilson (1980b) suggested that due to their hypervariable flower morphology, enormous species diversity within the genus was the result of repeated hybridization events. Though macromorphological characters are very useful for taxonomic identification of many plant species, yet these cannot be used to distinguish between kinds of closely related species that are morphologically indistinguishable but belong to different species. Therefore, to bring out morphological differences that may exist between closely related species, SEM analysis of micromorphological investigations of pollen grains are used.

A reticulate rectangular pollen grains in genus *Impatiens* with four apertures was first reported by Huynh in 1968. Janssens et al. (2012) investigated pollen morphology in 115 species and described as four-aperturate to tri-aperturate, and the polar view ranged from circular, quadrangular, elliptic, and sub-elliptic to rectangular. Yu et al. (2015) provided a new classification for the genus *Impatiens* based on morphological and molecular evidence.

Janssens et al. (2005) carried out a palynological study and variation was noticed from the results of pollen morphology. In their study, representative of samples of 11 African and 8 Asian taxa were used for observation using SEM, but the palynological evolution of Asian *Impatiens* species was unable to conclude. Janssens et al. (2018) characterized pollen morphology as circular, rectangular or elliptic and reticulate sexine ornamentation for most of the African *Impatiens* species. Lens et al. (2005)

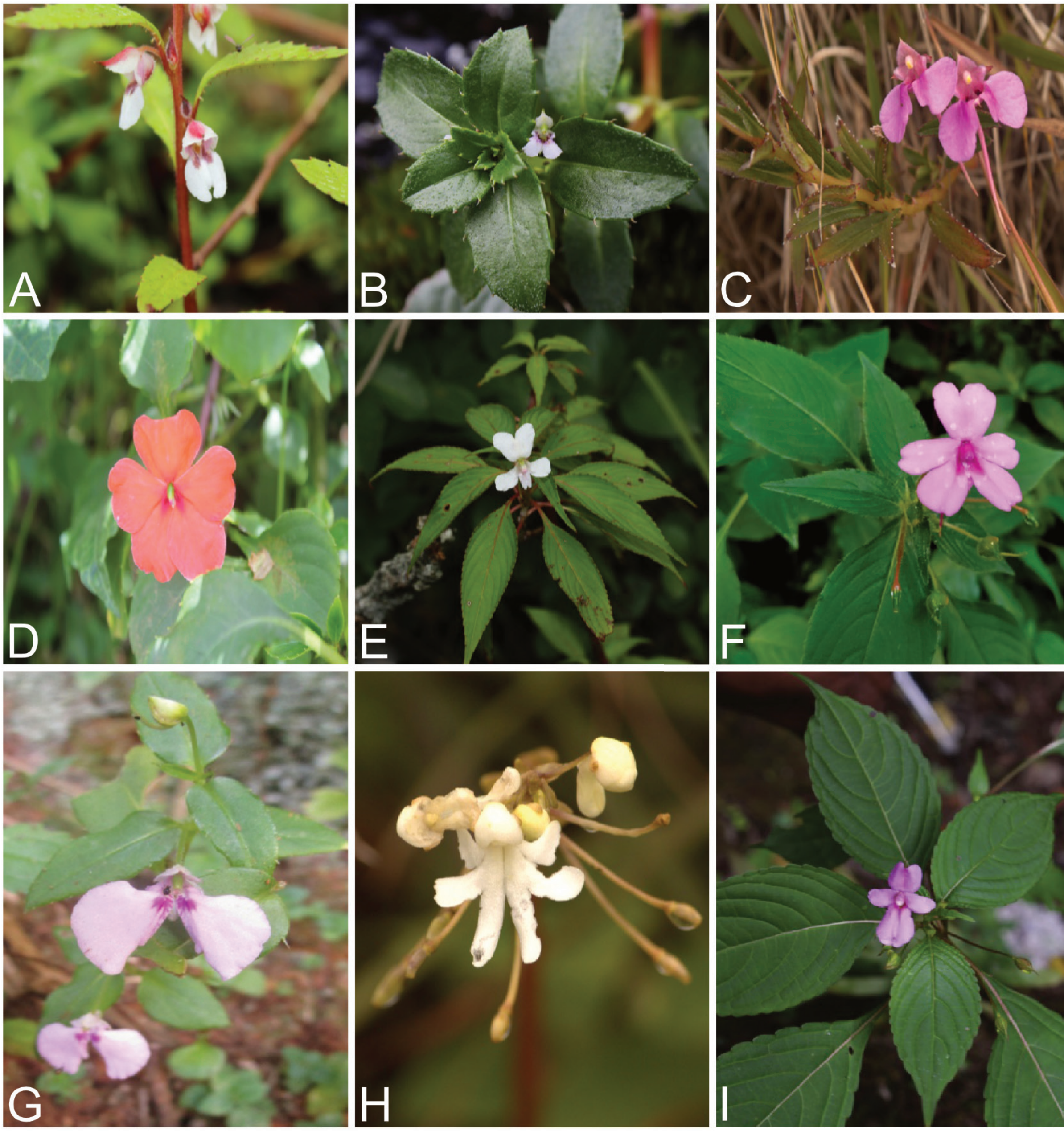

Figure 1. A: *Impatiens scabriuscula* Heyne; B: *Impatiens pendula* Heyne *ex* Wight & Arn.; C: *Impatiens fasciculata* Lam.; D: *Impatiens walleriana* Hook. f. E: *Impatiens cuspidata* Wight & Arn.; F: *Impatiens gardneriana* Wight; G: *Impatiens tenella* B. Heyne *ex* Wight & Arn.; H: *Impatiens clavicornu* Turcz.; I: *Impatiens latifolia* Wight.

carried out a detailed pollen morphological description of Balsaminaceae, Tetrameristaceae and Pellicieraceae by means of light microscopy, SEM and transmission electron microscopy revealed main aperture type in four-aperturate, colpate rectangular pollen grain with reticulate sexine ornamentation.

Bigazzi and Selvi (1998) suggested that palynological features are not changeable with environmental changes and have strong selection forces at work in dispersal, water-stress, pollination, germination, stigmatic interactions and possess strong taxonomic characteristics for species-genera identifications.

Taxonomically, *Impatiens* is notoriously difficult to classify due to the semi-succulent stems and fleshy leaves, flowers are extremely fragile, providing well-dried herbarium specimens is challenging and in dried specimens

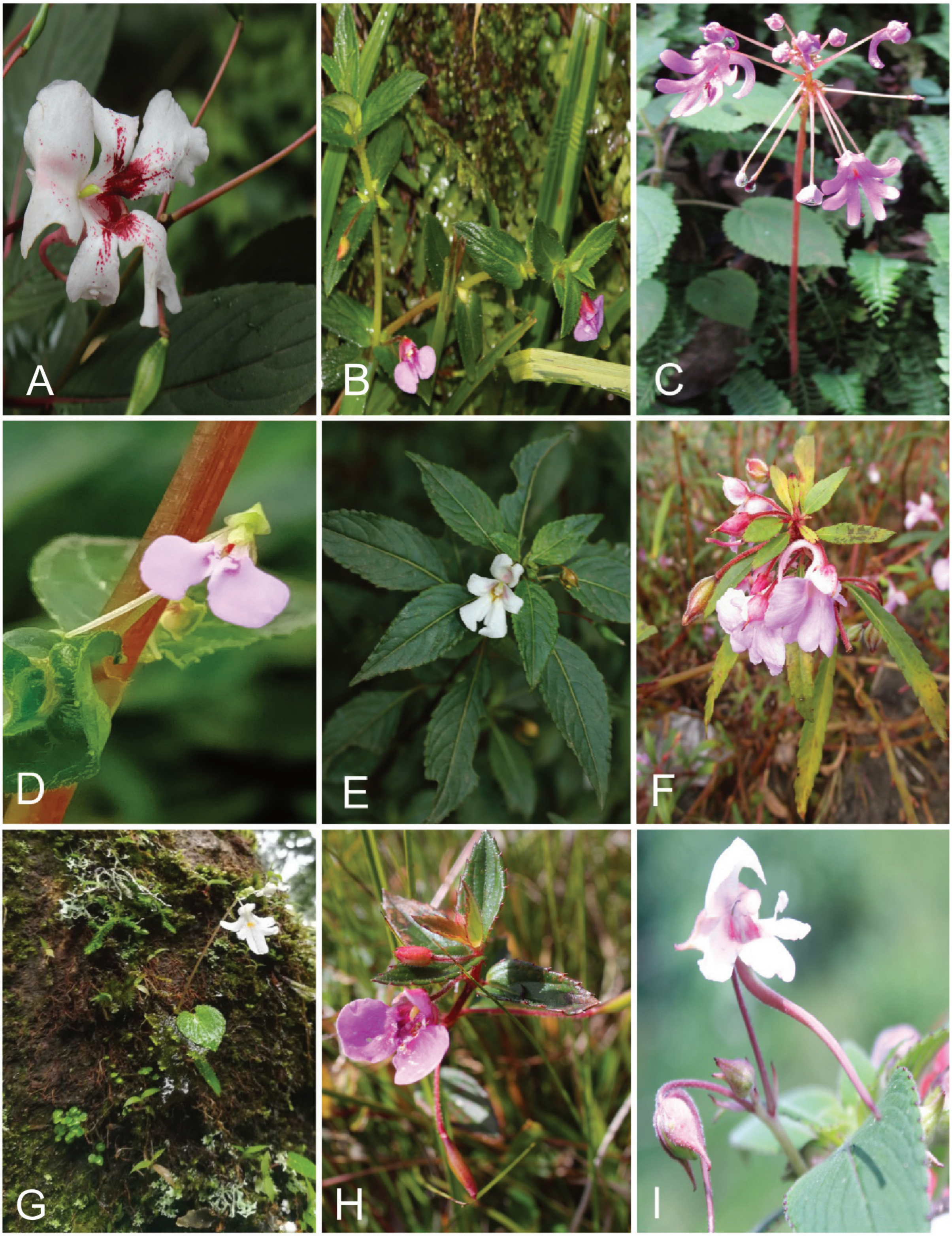

Figure 2. **A**: *Impatiens grandis* Heyne; **B**: *Impatiens oppositifolia* L.; **C**: *Impatiens levingei* Gamble *ex* Hook. f.; **D**: *Impatiens minor* (DC.) Bennet; **E**: *Impatiens leschenaultii* (DC.) Wall. *ex* Wight & Arn.; **F**: *Impatiens balsamina* L.; **G**: *Impatiens modesta* Wight; **H**: *Impatiens rufescens* Benth. *ex* Wight & Arn.; **I**: *Impatiens fruticosa* Lesch. *ex* DC.

most are folded and coalesced (Yu et al. 2015). Accordingly, Grey-Wilson (1980a, 1980b) suitable micromorphological characters to tackle the taxonomic complexity within the genus, as macromorphological characters have often proven unsuitable. Pollen grains can be retained in soil for a long period of time and its morphology is considered as conservative characters for plant classification because the shape of pollen grains specific to the taxonomic rank such as family, genus and species. Improved determination keys have been derived from extensive pollen analysis which could serve as a base for pollen studies by archaeologists, botanists, geologists, and immunologists (Erdtman 1986).

SEM offers distinct advantages for examination of unstained preparations of pollen and was used for the purpose of observing variations in the morphology of pollen of many plant taxa. Several studies (Lens et al. 2005; Janssens 2005, 2008, 2012) on the pollen morphology of *Impatiens* described their importance to understand the taxonomy of particular species. Even though several studies have been carried out, still the pollen morphology of this genus *Impatiens* require deeper understanding.

Material and methods

For palynological study, the specimens were collected along with its flower. Fresh pollen samples were collected from various localities of Nilgiris during the period of 2017-2019. The collected plant specimens were identified with the help of local floras (Gamble and Fischer 1915; Gamble 1921; Gamble 1934; Nair et al. 1983; Mattew 1991) and regional floras, revisions, monographs, and pertinent literature. The voucher specimens were deposited in the Herbarium of Department of Botany, Bharathiar University (BUH), Coimbatore. Further, the pollens were examined using scanning SEM.

The selected pollens were mounted directly on aluminum stubs using double sided adhesive tape and were sputter coated with in a thin layer of gold. Each taxon was studied for qualitative and quantitative character. SEM imaging was carried out with FEI Quanta 200 SEM (FELMI-ZFE, Graz, Austria) at the pollen laboratory. SEM micrographs were used mainly for studying the general shape, size, type of ornamentation, aperture characters and get more detailed information on the sculpturing.

Fifty pollen grains of each species were examined and an average measurement for the polar axis and equatorial axis, and lumen diameter were observed by ImageJ software. The P/E ratios were calculated. Pollen terminology follows the Punt et al. (2007). The terminology of pollen shape in polar view by the following Reitsma (1970). Terms for shape classes in equatorial view are adopted from Erdtman (1971). The ratio of polar length to equatorial length was calculated using the following formula.

The ratio of polar length to equatorial length = P/E

Where P denotes the diameter of the Polar axis and E denotes the diameter of the Equatorial axis.

Based on the palynological characters dichotomous taxonomic key for *Impatiens* was prepared. To evaluate the significant variations in quantitative character among the *Impatiens* taxa were determined by subjecting the data to one-way analysis of variance (ANOVA) using SPSS (version 16.0).

Results

The pollen morphology of 18 taxa of the genus *Impatiens* L. (Balsamiaceae) was investigated. A comprehensive description of the SEM data according to the pollen features in terms of the size, shape, shape in polar view, exine ornamentation, symmetry, polarity, polar length (P), equatorial length (E), the ratio of polar length to equatorial length (P/E) and the diameter of lumen measured for fifty pollen grains of each specimen are provided (Table 1 and 2).

All the collected species of *Impatiens* are monad. Most of the pollen grains were isopolar but certain species showed heteropolar grains, such as in *Impatiens fruticosa, Impatiens clavicornu, Impatiens levingei*, *Impatiens latifolia* and *Impatiens rufescens.* The pollen grain was radially symmetrical in *I. clavicornu*, *I. cuspidata*, *I. pendula, I. levingei*, *I. gardneriana, I. grandis*, *I. rufescens* and bilateral symmetrical in the remaining species. Selected SEM micrographs of examined pollen grains are presented in Fig. 3-6.

Size

According to size of the pollen grains it can be categorized into small size, large size and very large size (Kermp 1965). The pollen of the Nilgiris *Impatiens* species is generally small and medium sized. The small pollen grains were observed in *I. modesta, I. balsamina, I. pendula and I. rufescens* whereas the rest of the species are medium sized. The mean of the polar axis varies from 22.90 µm in *I. balsamina* to 36.19 µm in *I. walleriana*. The minimum equatorial axis (14.66 µm) is reported in *I. balsamina* and the maximum equatorial axis (28.52 µm) is reported in *I. gardneriana* (Table 1).

Shape in equatorial view

According to Erdtman (1952) the shapes of the pollen grains determine the ratio of polar axis and equatorial diameter. The highest P/E ratio was recorded in *I. walleriana* (1.78 µm) and the lowest was observed in *I. modesta*

Table 1. Dimensions and size variation in pollen grains of the *Impatiens* taxa.

Number	Taxon	Polar axis (P) µm		Equatorial axis (E) µm		P/E	Lumen diameter	Size
		Mean	Range	Mean	Range			
1	*I. balsamina L.*	22.90 ± 2.691^{i}	16.18 - 26.47	14.66 ± 1.913^{f}	10.12 - 18.59	1.56	1.20 ± 0.44^{g}	Small
2	*I. clavicornu* Turcz.	27.94 ± 2.810^{f}	21.61 - 33.34	24.29 ± 31.10^{bcd}	18 - 31.10	1.15	2.86 ± 1.00^{a}	Medium
3.	*I. cuspidata* Wight & Arn.	29.68 ± 2.608^{de}	24.50 - 34.21	22.34 ± 2.157^{cde}	16.08 - 27.75	1.32	1.80 ± 0.53^{def}	Medium
4.	*I. latifolia* Wight	31.07 ± 3.375^{c}	25.05 - 38.65	19.56 ± 2.174^{def}	16.36 - 23.23	1.58	2.11 ± 0.65^{bc}	Medium
5.	*I. fasciculata* Lam.	33.01 ± 2.273^{b}	27.61 - 38.09	22.19 ± 2.234^{cde}	16.45 - 26.87	1.48	1.68 ± 0.53^{ef}	Medium
6.	*I. fruticosa* Lesch. *ex* DC.	30.28 ± 2.491^{cd}	25.60 - 35.73	27.28 ± 2.287^{bc}	21.56 - 32.17	1.10	-	Medium
7.	*I. grandis* Heyne	27.73 ± 1.966^{c}	24.24 - 32.74	23.04 ± 2.235^{bc}	17.25 - 26.08	1.20	1.80 ± 0.52^{def}	Medium
8.	*I. gardneriana* Wight	31.46 ± 4.840^{c}	24.29 - 48.54	28.52 ± 3.729^{b}	22.38 - 35.25	1.10	2.20 ± 0.87^{b}	Medium
9.	*I. levingei* Gamble *ex* Hook. f.	25.94 ± 3.184^{g}	18.89 - 31.62	17.56 ± 2.265^{ef}	14.67 - 22.82	1.46	1.58 ± 0.44^{f}	Medium
10.	*I. leschenaultii* (DC.) Wall. *ex* Wight & Arn.	28.64 ± 3.860^{ef}	21.81 - 36.87	19.96 ± 2.911^{def}	16.39 - 28.31	1.43	1.91 ± 0.33^{cde}	Medium
11.	*I. modesta* Wight	23.10 ± 2.824^{i}	16.2 - 29.64	20.45 ± 3.053^{def}	15.18 - 28.19	1.09	1.96 ± 0.63^{bcd}	Small
12.	*I. minor* (DC.) Bennet	25.19 ± 2.586^{gh}	17.94 - 29.39	16.80 ± 2.455^{ef}	11.57 - 20.84	1.49	1.71 ± 0.56^{def}	Medium
13.	*I. oppositifolia* L.	28.67 ± 2.163^{ef}	24.26 - 33.45	19.68 ± 1.544^{def}	16.78 - 22.77	1.45	1.84 ± 0.53^{de}	Medium
14.	*I. pendula* Heyne *ex* Wight & Arn.	24.16 ± 2.400^{hi}	18.65 - 29.26	20.62 ± 2.689^{def}	14.16 - 24.57	1.17	1.19 ± 0.33^{g}	Small
15.	*I. rufescens* Benth. *ex* Wight & Arn.	23.83 ± 2.476^{i}	18.75 - 26.31	18.84 ± 2.260^{def}	14.73 - 20.92	1.26	1.28 ± 0.30^{g}	Small
16.	*I. scabriuscula* Heyne	28.36 ± 2.558^{f}	21.38 - 34.11	17.50 ± 2.637^{ef}	13.15 - 22.67	1.62	1.12 ± 0.34^{g}	Medium
17.	*I. tenella B.* Heyne *ex* Wight & Arn.	28.38 ± 2.862^{f}	20.76 - 33.42	17.38 ± 2.693^{ef}	12.69 - 23.56	1.63	1.58 ± 0.44^{f}	Medium
18.	*I. walleriana* Hook. f.	36.19 ± 4.634^{a}	28.06 - 44.18	20.24 ± 2.723^{def}	14.66 - 26.44	1.78	1.94 ± 0.63^{cde}	Medium
		F= 71.953* **		**F= 16. 522*** **			**F=52.023*** **	**Df-17,908**

Means ± Standard Error in a row followed by a same letter(s) are not significantly (P > 0.05) different according to Duncan's Multiple Range Test.
Df- Degree of freedom
***= Significant at 0.001% level

Table 2. Details of pollen morphological characteristics in selected species of *Impatiens*.

Number	Taxon	Shape	Polar view	Sexine ornamentation	Symmetry	Polarity	Number of aperature
1	*I. balsamina L.*	Prolate	Rectangular	Reticulate	Bilateral	Isopolar	Dicolpate
2	*I. clavicornu* Turcz.	Subprolate	Circular	Reticulate	Radial	Heteropolar	Tricolpate
3.	*I. cuspidata* Wight & Arn.	Subprolate	Elliptic - circular	Reticulate	Radial	Isopolar	Monocolpate
4.	*I. latifolia* Wight	Prolate	Elliptic	Reticulate	Bilateral	Heteropolar	Monocalpate
5.	*I. fasciculata* Lam.	Prolate	Elliptic	Reticulate	Bilateral	Isopolar	-
6.	*I. fruticosa* Lesch. *ex* DC.	Prolate - spheroidal	Quadrangular	Echinate	Bilateral	Heteropolar	-
7.	*I. grandis* Heyne	Subprolate	Elliptic	Reticulate	Radial	Isopolar	-
8.	*I. gardneriana* Wight	Prolate - spheroidal	Elliptic - circular	Reticulate	Radial	Isopolar	-
9.	*I. levingei* Gamble *ex* Hook. f.	Prolate	Triangular	Reticulate	Radial	Heteropolar	Tricolpate
10.	*I. leschenaultii* (DC.) Wall. *ex* Wight & Arn.	Prolate	Rectangular	Reticulate	Bilateral	Isopolar	-
11.	*I. modesta* Wight	Prolate - spheroidal	Circular	Reticulate	Bilateral	Isopolar	-
12.	*I. minor* (DC.) Bennet	Prolate	Rectangular	Reticulate	Bilateral	Isopolar	-
13.	*I. oppositifolia* L.	Prolate	Elliptic	Reticulate	Bilateral	Isopolar	-
14.	*I. pendula* Heyne *ex* Wight & Arn.	Subprolate	Circular	Reticulate	Radial	Isopolar	-
15.	*I. rufescens* Benth. *ex* Wight & Arn.	Subprolate	Quinquangular	Reticulate	Radial	Heteropolar	Monocolpate
16.	*I. scabriuscula* Heyne	Prolate	Rectangular	Reticulate	Bilateral	Isopolar	Bicolpate
17.	*I. tenella B.* Heyne *ex* Wight & Arn.	Prolate	Rectangular	Reticulate	Bilateral	Isopolar	-
18.	*I. walleriana* Hook. f.	Prolate	Rectangular	Reticulate	Bilateral	Isopolar	Tetracolpate

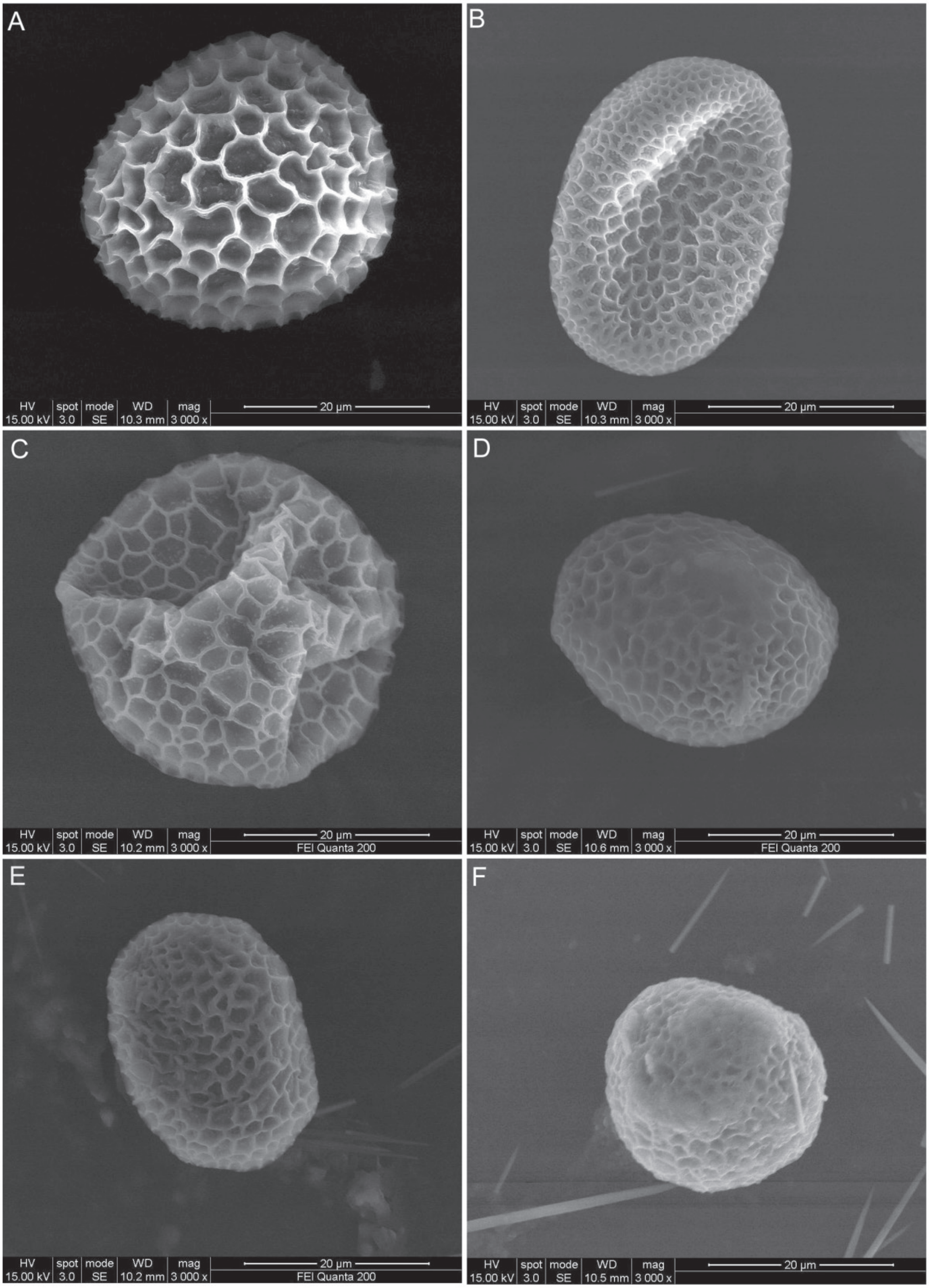

Figure 3. A: *Impatiens clavicornu* Turcz.; **B**: *Impatiens fasciculata* Lam.; **C**: *Impatiens gardneriana* Wight; **D**: *Impatiens grandis* Heyne; **E**: *Impatiens leschenaultii* (DC.) Wall. *ex* Wight & Arn.; **F**: *Impatiens pendula* Heyne *ex* Wight & Arn.

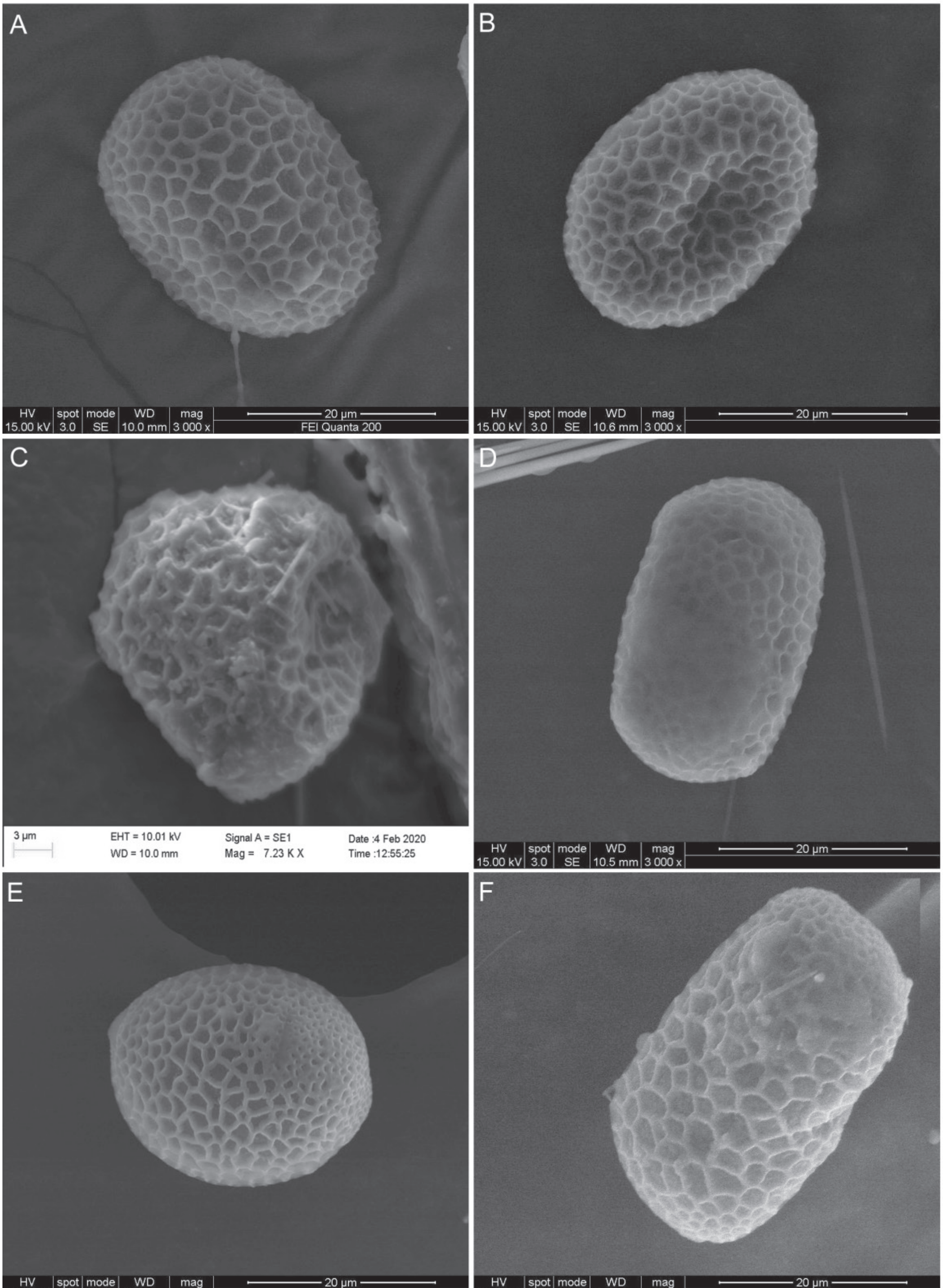

Figure 4. A: *Impatiens minor* (DC.) Bennet; **B**: *Impatiens oppositifolia* L.; **C**: *Impatiens rufescens* Benth. *ex* Wight & Arn.; **D**: *Impatiens tenella* B. Heyne *ex* Wight & Arn; **E**: *Impatiens cuspidata* Wight & Arn; **F**: *Impatiens walleriana* Hook. f.

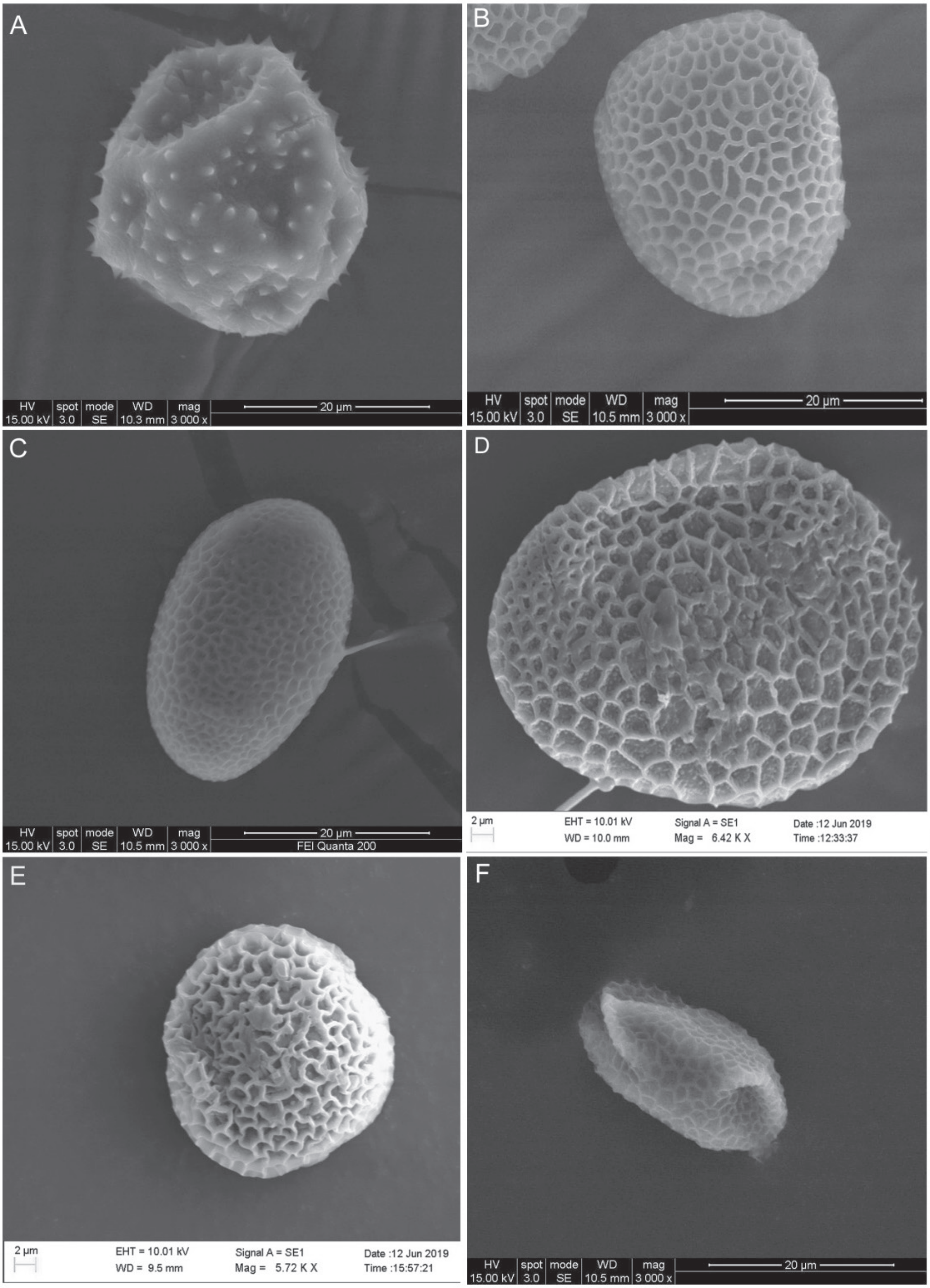

Figure 5. A: *Impatiens fruticosa* Lesch. *ex* DC.; **B**: *Impatiens levingei* Gamble *ex* Hook. f.; **C**: *Impatiens scabriuscula* Heyne; **D**: *Impatiens latifolia* Wight.; **E**: *Impatiens modesta* Wight; **F**: *Impatiens balsamina* L.

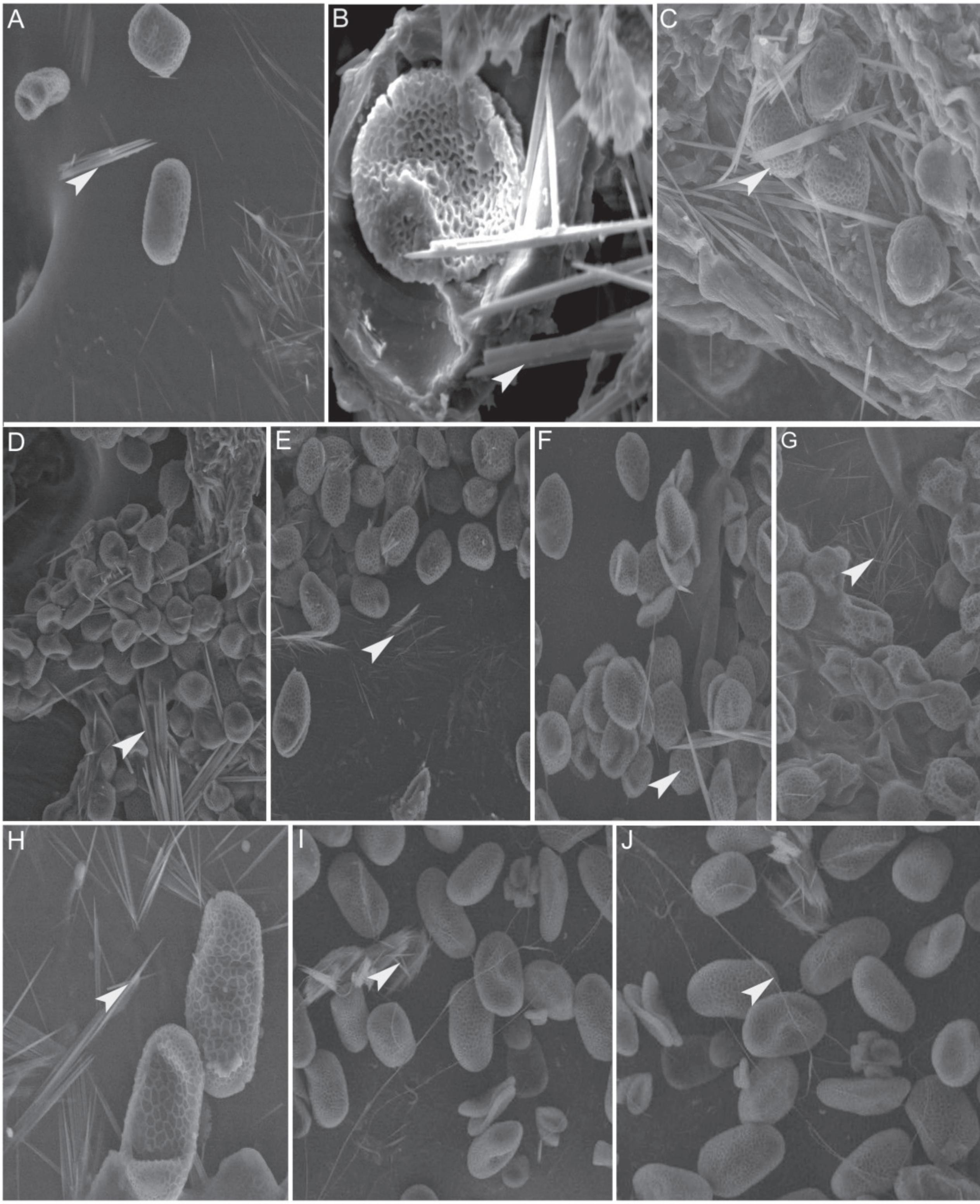

Figure 6. A-I Shows the rapids in pollen grains. **A**: *Impatiens tenella* B. Heyne *ex* Wight & Arn.; **B**: *Impatiens rufescens* Benth. *ex* Wight & Arn.; **C**: *Impatiens pendula* Heyne *ex* Wight & Arn.; **D**: *Impatiens minor* (DC.) Bennet; **E**: *Impatiens leschenaultii* (DC.) Wall. *ex* Wight & Arn.; **F**: *Impatiens grandis* Heyne; **G**: *Impatiens gardneriana* Wight; **H**: *Impatiens walleriana* Hook. f. **I**: *Impatiens scabriuscula* Heyne. **J:** pollen thread in *Impatiens scabriuscula*.

(1.09 µm). Most of the examined pollen shape is prolate (10 spp.) or subprolate (5 spp.) and prolate-spheroidal (3 spp.), respectively (Table 2).

Shape in polar view

The present study observed a wide range of pollen shape within the Nilgiris *Impatiens* species, varying from rectangular, circular, elliptic, triangular, elliptic-circular and quadrangular of all the studied *Impatiens*. The shape of majority of the pollen grains (6 spp.) of genus *Impatiens* are rectangular. Some others are elliptic (4 spp.) and about 2 species are elliptic-circular. Alternatively, quadrangular, triangular, quinquangular shapes have also been observed from negligible number (1) of species. Erdtman (1952) stated that the shape of the pollen grains determines the ratio of polar axis and equatorial diameter. The present study recorded the highest P/E ratio in *I. walleriana* (1.78) and the lowest was observed in *I. modesta* (1.09) (Table 1).

Exine ornamentation

The reticulate exines ornamentation was observed in most of the *Impatiens* species except for *I. fruticosa* which showed a rather distinct echinate type of ornamentation. The highest lumen diameter was recorded in *I. clavicornu* (2.86 µm) and lowest was observed in *I. scabriuscula* (1.12 µm).

Aperture characters

The apertures observed within *Impatiens* are simple and mostly monocolpate, dicolpate, tricolpate and tetracolpate. The colpate aperature is monocolpate with four taxa (*I. scabriuscula, I. rufescens, I. latifolia* and *I.cuspidata*), dicolpate with one taxa (*I. balsamina*), tricolpate with 2 taxa (*I. levingei* and *I. clavicornu,*) and tetracolpate is single taxa (*I. walleriana*), respectively. It is clear from our data that the aperture condition greatly defines pollen shape; the number of aperture and polar view also should be considered as dependent characters (Janssens et al. 2012).

Raphides

In all *Impatiens* species examined, there are some polynological features that are rare in the species, especially in case of *I. scabriuscula, I. walleriana, I. tenella, I. rufescens, I. pendula, I. minor, I. leschenaultii, I. grandis* and *I. gardneriana.* They could easily be distinguished from other species by the presence of raphides.

Pollen threads were observed in only mature anthers of *I. scabriuscula* shown in Figure 6 as white arrowheads in photo J but are present in the majority of the Asian *Impatiens* (Vogel and Cocucci 1988). These fine cellulose fibers form a dense network covering the anther slits. Most of the grains have granules in the foot layer in the lumina at the inside of the lumina. Granules can be solitary or interconnected and fused with neighboring muri. Although, rarely present in *Impatiens* a few south Indian species with a reticulate ornamentation pattern are characterized by a microreticulate margo in *I. cuspidata.*

Discussion

Palynology of genus *Impatiens* as described in Table 3. are generally prepared for observing the characteristic feature of *Impatiens* species under the SEM without disturbing the characteristic morphological features. For such characterization, the following features of pollen grains, pollen shape and exine ornamentation were observed which might greatly help in the identification of the genus *Impatiens* under study. It is apparent from other related studies that routine morphological feature assessment does not always give complete proof or identity of species.

Presence or absence of raphides was also differed from one another thus they might appear differently to view under SEM technique. Kubilzki (2004a, b); Lens et al. (2005) considered all subclades of balsaminoids were found to have the features of raphides, grouped under the Ericales. Pollen threads were observed in only mature anthers of *I. scabriuscula* but are present in the majority of the Asian *Impatiens* (Vogel and Cocucci 1988).

Categorizing genus *Impatiens* into different groups based on colporate alone does not however reflect their complete variation. It also adds that pollen grains are microscopically versatile requiring wide range of variables at polarity, symmetry, sexine ornamentation, shape and polar view as differentiating factors. These features have been observed for the monocolpate to biological characteristics which can be an aid to identification of species of *Impatiens*. In number of apertures, pollen grains of genus *Impatiens* are monocolpate to tetracolpate. Certain species are however no colpate.

The size of the pollen grains facilitated the differentiation of pollen grain with small as well as medium under SEM. Because of only small and medium size variations involved in the size for the differentiation of pollen grains, attention has been focused on the study of equatorial axis of pollen grains. The equatorial axis less than 14.66 (µm) has not been observed in Asian *Impatiens* under SEM. Majority of pollen grains of *Impatiens* species are smaller than 28.52 µm. The mean average E-axis of Asian *Impatiens* was about 16.522 µm.

Because of the variation on the polar axis, they are being classified as rectangular, quadrangular, elliptic, circular, triangular, etc. Majority of the large sized pollen grains are rectangular and elliptic circular type. The heterogeneity in the shape of pollen grain in genus Impatiens implies that distinct shapes of prolate, subprolate, prolate spheroidal are evident. The pollen grains of *I. gardneriana*

Wight exhibited the highest equatorial axis with the elliptical circular polar axis and prolate spheroidal shape. The pollen grains of African genus *Impatiens* on the other hand possess no prolate pollen (Janssens et al. 2012). The results of the present study are not in accordance with the findings of Janssens et al. (2012). They reported the pollen grains of genus *I. leschenaultii* were quadrangular in shape. But the present study revealed the shape was rectangular.

Pollen grains of Asian *Impatiens* are generally monocolpate. Bi-, tri- and tetracolpate are also present in Asian *Impatiens*. Perveen and Qaiser (2001) recognized tetracolpate, rectangular and reticulate from Pakistan. Grey-Wilson (1980a) was the first to stress the possible value of pollen characters to tackle taxonomical questions in *Impatiens* as he used pollen morphological data to detect possible hybrids and to optimize his species aggregation hypothesis on African *Impatiens*.

The pollen grains of Asian *Impatiens* are aporus and the pollen grains of African *Impatiens* on the other hand possessed frequently distributed pori. This is supportive in agreement to the results observed by Huynh (1968); Grey-Wilson (1980b). The sexine ornamentation of the Asian *Impatiens* shows reticulate type and plesiomorphic. The lumen size of Asian *Impatiens* varies considerably among clades or even between closely related species. One species was Echinate (*I. fruticosa* Lesch. *ex* DC). Similarly, different species of Asian *Impatiens* such as *I. cyclocoma, I. javensis, I. hirsuta* and *I. fruticosa*) were found to have echinate sexine ornamentation.

Ruchisansakun et al. (2015) stated that the combination of molecular phylogenetic and morphological character has facilitated the delimitation of natural infrageneric lineage within the complex genus *Impatiens* and suggested that even though molecular species demonstrated the critical nature of previous *Impatiens* classification, new monophyletic and clearly diagnosable lineages can be found in *via* extended character state research. Yu et al. (2015) analyzed the pollen, seed morphology and phylogeny of *Impatiens* using the three molecular markers such as ITS, *atpB* and *trnL-F*.

Pollen grains are generally four-aperturate. Tri-aperturate pollen is also present in African balsams but less frequent compared to four-aperturate pollen (Janssens et al. 2012). Balsaminaceae that shows a distinct variation is the granule density within the lumina or they can be fused with each other forming a dense mass. Another pollen character within Balsaminaceae shows a distinct variation as the granule density in the lumina (Janssens et al. 2005). Granules can either be absent or very sparse or they can be fused with each other and with neighboring muri. In some cases, granules fill nearly the entire lumina (Janssens et al. 2018). According to recent analysis (Janssens 2006, 2007, 2009b) these species are closely related thereby suggesting that the presence or absence of a margo might be a taxonomically useful character within Asian *Impatiens*.

Key to species based on pollen morphology

1a. Sexine echinate	**6. *I. fruticosa***
b. Sexine reticulate	2.
2a. Shape prolate	3.
b. Shape subprolate	12.
3a. Radial symmetry	**9. *I. levingei***
b. Bilateral symmetry	4.
4a. Heteropolar	**4. *I. latifolia***
b. Isopolar	5.
5a. Pollen size small	**1. *I. balsamina***
b. Pollen size medium	6.
6a. Polar axis < 35 µm	**18. *I. walleriana***
b. Polar axis > 35 µm	7.
7a. Aperturate	**16. *I. scabriuscula***
b. Non aperturate	8.
8a. Lumen diameter <1.85 µm	**10. *I. leschenaultii***
b. Lumen diameter >1.85 µm	9.
9a. P/E ratio >1.8 µm	**13. *I. oppositifolia***
b. P/E ratio <1.8 µm	10.
10a. Polar view elliptic	**5. *I. fasciculata***
b. Polar view rectangular	11.
11a. Equatorial view <17µm	**17. *I. tenella***
b. Equatorial view >17 µm	**12. *I. minor***
12a. Polar view quinquangular	**15. *I. rufescens***
b. Polar view not quinquangular	13.
13a. Heteropolar	**2. *I. clavicornu***
b. Isopolar	14.
14a. Pollen size small	**14. *I. pendula***
b. Pollen size medium	15.
15a. Colpate- 1	**3. *I. cuspidata***
b. Colpate-2	16.
16a. Prolate-spheroidal	17.
b. Other than prolate-spheroidal	**7. *I. grandis***
17a. Radial symmetry	**8. *I. gardneriana***
b. Bilateral symmetry	**11. *I. modesta***

Conclusion

The result concluded that the peculiar characteristics of *Impatiens* pollen are medium sized, quadrangular, reticulate and exine ornamentation. The palynological features may appear as tough to differentiate species but in reality, they are fascinating and quite useful for classification and identification of closely related species in a genus. The lack of simplified, illustrated SEM analysis is the major constrain experienced by several systematic researchers. Palynological feature through SEM differentiation is the best procedure to justify the species and phylogenetic relationship. The present study, in addition with the reference from the previous literature confirmed the pollen morphological character through SEM is inevitable for the taxonomic identification of the species and it will be the base line information resolving many taxonomical problems on evolutionary relationship of plant species grouped under respective families. Therefore, SEM analysis is a valuable tool for studying the pollen morphological features for the species identification of genus *Impatiens*.

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